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Receiving element for receiving a specimen detached from a  
biological mass by means of laser radiation

The present invention relates to a receiving element for

5 receiving a biological specimen which has been detached,  
and in particular catapulted out, i.e. detached by  
transmission of a momentum, from a biological mass by means  
of laser radiation.

10 WO 97/29355 A from the present applicant proposes a novel  
method for sorting and recovering individual biological  
specimens which are arranged on a carrier. In particular,  
it is proposed in said publication to separate a previously  
selected biological specimen from the surrounding further  
15 biological mass by a laser beam, such that the selected  
biological specimen is dissected out of the further  
biological mass. The dissected out biological specimen is  
then catapulted with the assistance of a laser pulse from  
the carrier to a collecting device where it is collected  
20 and held by a collecting or receiving element, in  
particular in the form of a pot-shaped container. With  
appropriate adjustment of laser power and/or laser focal  
point, it is also possible to catapult the selected  
biological specimen directly out of the surrounding  
25 biological mass with the assistance of just one single  
laser pulse, such that separate laser irradiation for  
cutting out the desired biological specimen is not  
necessary.

30 For the purposes of the present patent application,  
"biological specimens" are generally taken to mean any  
biological cells, in particular in a live or immobilised  
state, or cellular constituents which are a constituent of  
a liquid or solid biological material, such as for example

of a cellular tissue, microtome preparations thereof, of a swab sample or of a cell culture etc..

The present invention is described below with reference to  
5 the field of application of processing biological  
specimens.

Figure 3 shows the structure of a laser-microscope system  
as may be used with an above-described collecting device or  
10 an above-described receiving or collecting element. The  
system is of modular structure and may thus be individually  
adapted to different experimental requirements.

The system shown in Figure 3 comprises a laser device 17,  
15 in which a laser light source for generating a laser light  
beam is accommodated. Furthermore, a lens system 15, 16 is  
accommodated in the laser device 17, which system is  
necessary for coupling the laser beam into a microscope 13  
and adjusting the laser focal point in the specimen plane  
20 to the optical focal point of the microscope 13. In the  
present case, the laser may comprise a pulsed UV nitrogen  
laser. A control panel may be provided in order to control  
the laser device 17, with the assistance of which panel  
laser power and/or laser focal point may be set to desired  
25 values. A quartz filter 15 is arranged perpendicularly to  
the laser beam path for precise adjustment of laser power,  
the position of which filter may be controlled as a  
function of the adjustments made on the control panel in  
order to adjust laser power accordingly. The quartz filter  
30 5 may here be adjusted both automatically and manually. In  
addition to adjustment of laser power, the laser focal  
point may also be adjusted independently of the microscope  
focal point, i.e. the focal point of the laser may be  
displaced in the Z direction relative to the specimen plane

of the microscope 13. The laser focal point may also be adjusted both automatically and manually as a function of the adjustments made on the control panel by a corresponding movement of the lenses 16. The pulse rate of 5 the laser may preferably also be adjusted by means of the stated control panel, the adjustment made on the control panel being displayed.

The laser beam is coupled into the microscope 13 via a 10 plurality of coated beam splitters and deflected to an objective 12. The laser beam emitted via the objective 12 finally impinges on a motorised and computer-controlled microscope or specimen stage 14, on which is arranged a microscope slide with a biological mass to be processed. 15 Above the specimen stage 14 there is located a likewise motorised and preferably computer-controlled collecting device 19, which comprises one or more receiving or collecting elements or collecting vessels 1. Components 14 and 19 permit exact specimen positioning and precise 20 collection of biological or nonbiological specimens which are catapulted out and upwards from the mass located on the specimen stage 14 by means of laser irradiation.

The microscope 13 may be of any desired design. In 25 particular, it is in principle conceivable to use not only an inverted microscope (as shown in Figure 3) but also an upright microscope or a laser microscope. The microscope 13 is equipped with a video camera which photographs the area of the microscope slide or specimen stage 14 above the 30 objective 12. The video signal of this video camera is supplied to a conventional commercial computer 18 and subjected therein to image processing in such a manner that the corresponding video image may be displayed in real time on the screen or monitor 8 of the computer 18.

In the computer 18 or the software running thereon, various functions are implemented which permit computer-assisted, i.e. automatic, actuation both of the laser device 17 and 5 also of the microscope 13 or of the specimen stage 14 and the collecting device 19, such that for example the laser is automatically activated and the collecting device 19 and the specimen stage 14 may be automatically displaced and adjusted. Conventional input means, such as for example a 10 keyboard 9, a computer mouse 10 or (not shown) a trackball, joystick or the like are provided to adjust or select these functions. Furthermore, a foot switch 11 is assigned to the laser device 17, actuation of which switch allows manual activation of the laser.

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In order to cut the biological mass located on the microscope slide or the specimen stage 14, the user may, with computer assistance, specify a suitable cutting line which is implemented by corresponding actuation of the 20 laser device 17 and of the specimen stage 14 in a corresponding relative motion between the laser beam and the specimen stage 14, such that simultaneous activation of the laser device 17 effects cutting of the biological mass in accordance with the predetermined section line by means 25 of the laser beam.

A specimen cut out in this manner from the biological mass can be catapulted with the assistance of further laser irradiation out of the biological mass to the collecting 30 device 19 located thereover. To this end, the specimens to be catapulted may be defined or marked with computer assistance and then the specimen stage 14 may be adjusted automatically in such a manner that the specimens to be catapulted are moved in succession over the laser beam and,

by initiation of a short laser pulse, also known as a laser shot, in each case catapulted out of the specimen plane to the collecting device 19. Apart from the above-described automatic production of a laser pulse an individual laser 5 impulse or laser pulse may also be initiated by brief pressure on the foot switch 11 shown in Figure 3.

As has already been mentioned above, it is in principle also possible to catapult individual specimens directly out 10 of the surrounding biological mass by appropriate laser irradiation if laser power and/or laser focal point are appropriately adjusted such that prior cutting out is then no longer required.

15 The collecting device 19, which in the inverted system shown in Figure 3 is located above the specimen stage 14 or the specimen plane, comprises one or more receiving or collecting elements which collect and then retain the specimen catapulted out from the specimen plane. By 20 focussing the microscope 13 on the collecting device 19 or the receiving or collecting element 1 which is in each case located in the light path of the microscope 13, the biological or nonbiological specimen which has been catapulted out and held by the corresponding receiving or 25 collecting element may then be observed and investigated via the microscope 13 or the screen 8 of the computer 18, wherein for this purpose adjusting means are preferably provided for adjusting the collecting device 19 parallel to the specimen plane in order, using the microscope 13, to be 30 able to scan the specimen which has been catapulted out and held in the corresponding receiving or collecting element 1.

It should be noted that the distance between the collecting device 19 and the carrier 14 is not to scale in the drawings. It is desirable here to provide the smallest possible distance in order to permit precise collection of 5 the catapulted out specimens.

In order to ensure secure adhesion of the catapulted out specimen to a receiving surface of the receiving element 1, it is known to provide such a receiving surface with an 10 adhesive layer. However, the problem then often arises of detaching the specimens from this adhesive layer for further processing without causing damage. In addition, for example when a person touches the receiving element, electrostatic forces may arise which attract the specimen 15 as a result of which the specimen does not correctly reach the receiving surface. Such electrostatic charging may also be produced by the action of the laser beam. In order to avoid this, a liquid is conventionally placed in the receiving unit which is intended to prevent electrostatic 20 forces from arising. This has the disadvantage that this liquid must be introduced beforehand, which takes time and entails a risk of contamination. In addition, such a liquid may evaporate and must accordingly be introduced into the receiving unit relatively shortly before catapulting, which 25 may be problematic in multiple receiving units, such as for example microtitre plates.

Furthermore, there is the risk with conventional adhesive agents that difficulties may arise in detaching the 30 specimen from the adhesive agent without destroying it.

For further processing of the specimens, for example for recovering a cell culture or a biological mass, it may be necessary to add for example nutrient solutions or other

agents for further processing. This again gives rise to an elevated risk of contamination.

It is accordingly an object of the present invention to

5 provide a receiving unit which avoids at least some of the stated problems. In particular, it is desirable to be able to produce such a receiving unit in advance, for example for mass processing of biological material and to be able to carry out further processing of catapulted out or

10 detached specimens in as sterile a manner as possible.

Said object is achieved by a receiving element according to claim 1, 6, 7 or 8. The dependent claims define preferred exemplary embodiments of the receiving element, a preferred

15 use of the receiving element and a method for recovering a biological specimen using the receiving element.

The present invention provides a receiving element for receiving a specimen detached from a biological mass by

20 means of laser radiation, the receiving element comprising a receiving surface for receiving the specimen and the receiving surface comprising an adhesive agent for enhancing the adhesion of the respective specimen to the receiving surface. This adhesive agent is designed

25 according to the invention such that it suppresses the occurrence electrostatic forces, acting on the specimen, in the receiving element. According to a further aspect of the invention, the adhesive agent may be dissolved without damaging the specimen, for example may be liquefied by

30 input of heat. "Without damage" means in this context that predetermined processing and/or analysis of the specimen is not impaired. In addition or alternatively, the adhesive agent may be designed in such a manner that it can accommodate agents for further processing and/or analysis

of the specimen, such as for example a buffer solution, a nutrient solution and/or enzymes or enzyme prebatches for example for a polymerase chain reaction (PCR). It is of course particularly preferred to provide an adhesive agent  
5 which comprises two or all three of these features.

The invention furthermore provides a receiving element of this generic type in which the adhesive agent is a hydrogel. This hydrogel may in particular exhibit the  
10 above-stated properties. A hydrogel is generally taken to mean a gel based on hydrophilic, but water-insoluble polymers, which in particular assume the form of a three-dimensional network. The polymers may here be both natural polymers and synthetic polymers. One possible such hydrogel  
15 is agarose.

In a preferred exemplary embodiment, the receiving element comprises a lid portion for covering a container and a base portion fitted in the lid portion with the receiving  
20 surface on a side remote from the lid portion. The base portion is here preferably of a height which is so selected that the distance from the receiving surface to a base of the container in a state in which the lid portion is covering the container is less than 10 mm and preferably  
25 between 1 and 3 mm. If a laser radiation-transmitting container, in particular a Petri dish with double membrane base, is used as the container, said container may then be directly used as a carrier for the biological mass and the specimen may simply be catapulted onto the receiving  
30 surface when the lid portion is covering the container.

The receiving element may also take the form of a multiple culture dish, Petri dish or a microtitre plate in each case

having a plurality of receiving wells, the receiving wells being filled up to a specific height with the hydrogel.

The present invention is explained in greater detail below 5 with reference to the attached drawings and to preferred exemplary embodiments. In the drawings:

Fig. 1 shows a first exemplary embodiment of the present invention,

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Fig. 2 shows a second exemplary embodiment of the present invention, and

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Fig. 3 shows a laser system in which the present invention may be used.

Fig. 1 shows a first exemplary embodiment of the invention. A receiving element 1 according to the invention here comprises a cover or a lid 2 of a cell culture dish 5, for 20 example a Petri dish. A base or supporting element 3 is fitted on the inside of the lid 1. The supporting element 3 may here for example be circular and be made from silicone or acrylic polymer, other materials also being conceivable. For many applications, it is desirable for this supporting 25 element 3 to be autoclavable so that the receiving element may be sterilised. For this purpose, the supporting element 3 is sterilised and the agarose layer 4 applied by casting high-percentage, sterilised, filtered agarose into an appropriate template. The supporting element 3 is attached 30 by a non-toxic material to the inner surface of the lid 2, wherein this connection is reversible so as to be able to remove the supporting element 3 again if required.

On a lower side of the supporting element 3 there is a receiving surface for in particular biological specimens, which is covered with an agarose layer 4 preferably consisting of high-quality, i.e. high purity LE agarose.

5 The agarose layer serves as an adhesive agent for securing the specimens to the receiving surface. In principle, another suitable hydrogel or another adhesive agent with the desired properties may also be used instead of the agarose layer 4.

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For the catapulting process described in detail with reference to Fig. 3 in the introduction to the description, the lid 2 is placed on a suitable Petri dish 5. Instead of a conventional glass base, the Petri dish 5 preferably 15 comprises a double membrane 5a as base element, which consists of a combination of a light-transmitting and a UV-absorbing film. Such a Petri dish is described in detail in DE 100 39 979 A1. This Petri dish 5 contains a cell culture 7 for example on the double membrane 5a. When assembled, 20 the distance between the receiving surface or the hydrogel 4 and the membrane 5a is less than 10 mm, preferably in the range from 1 to 3 mm. The vessel closed in this way is then inserted as carrier 14 into the apparatus of Fig. 3. Using a laser beam, a desired part of the cell culture 7 is cut 25 out and catapulted by means of a laser pulse onto the hydrogel 4. The receiving element 1 thus at the same time replaces a collecting means 19 as illustrated in Fig. 3.

For further processing, the receiving element 1 is then 30 placed for example on a cell culture vessel or indeed on a further Petri dish 5 with membrane 5a. The cells catapulted onto the hydrogel 4 may be detached by gentle motion or by heating the agarose layer 4. The further Petri dish 5 may in particular be filled with a cell culture liquid, into

which the hydrogel 4 dips. Alternatively, the hydrogel layer may also be completely dissolved by the addition of agarase, an enzyme which dissolves agarose. Then the receiving element 1 may be replaced with a conventional lid 5 and reused, optionally after sterilisation. If the catapulted cells are transferred in this way into a further Petri dish 5 with membrane 5a, the process may be repeated after culturing in this Petri dish with the cell cultures which arise therein.

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Use of the hydrogel, in this case the agarose layer 4, has the advantage that electrostatic forces produced for example by the laser irradiation or by contact do not result in the cells which have been catapulted out 15 remaining attached to the lid 2 instead of on the hydrogel layer 4.

Another advantage of using such a receiving means is that the agarose layer 4 allows better visualisation of the cell 20 culture 7 and the catapulted-out cells with a microscope, since contrast is improved.

Fig. 2 shows a second exemplary embodiment of the present invention. Here, the receiving element 1 takes the form of 25 so-called multiple culture dishes, i.e. a plurality of connected dish-like wells 20. In the example illustrated, three such dishes 20 are shown, these being connected by webs 6. The dishes may be covered with a lid (not shown). With conventional commercial multiple culture dishes or 30 multiple culture plates, substantially more of these dishes 20 are conventionally present, for example four rows each with six such dishes 20. The dishes are again filled with agarose 4 or another hydrogel. In a catapulting process as described above, this arrangement is accordingly fitted

with its opening pointing downwards into the receiving means 19 of the microscope illustrated in Fig. 3, and the desired cells are catapulted onto the agarose layer 4 from a carrier 14. The level to which the dishes are filled with 5 agarose is so selected that the spacing relative to the carrier 14 is favourable to catapulting, being preferably between 1 and 3 mm.

After catapulting, the agarose 4 may again be liquefied by 10 the addition of agarase, such that immediate re-use is possible. Moreover, different additives such as for example cell culture media or buffer solutions may be introduced in the agarose layers 4. For subsequent processing or analysis 15 of the catapulted cells, additional introduction of these agents is therefore no longer necessary, since they are liberated once the agarose has been dissolved or liquefied. Enzymes or enzyme prebatches may in this case also serve as additives. One operation is therefore dispensed with, so avoiding the associated risk of contamination, since these 20 additives do not have to be added for example by pipetting, and the desired processing and/or analysis may be initiated immediately. One preferred field of application is in this case the polymerase chain reaction (PCR), wherein the additives may preferably be so selected that the reaction 25 is initiated at the same time as dissolution of the hydrogel by heating. Another field of application is culturing of the catapulted cells.

In such applications, care must be taken to ensure that the 30 dissolved hydrogel does not interfere with or contaminate the intended processing or analysis. High purity, preferably sterilised agarose is here too an example of a suitable hydrogel.

Instead of multiple culture dishes, so-called microtitre plates or 96-well microtitre plates may also be filled, as in Fig. 2, with fine-pore, finely gelling agarose, such that once again optimum spacing is provided for a

5 catapulting process. Here too, mixtures desired for further processing of the catapulted cells may again be admixed with the agarose, for example reaction mixtures such as denaturing buffer solutions or enzyme-containing prebatches. Thus, a desired reaction may be initiated for

10 all harvested cells at the same time. Since the agarose may be dissolved by the agarase enzyme, the full volume of the respective wells is available for subsequent applications such as PCR or MALDI (polymerase chain reaction or matrix-assisted laser desorption/ionisation). In such

15 applications, instead of dissolution of the agarose by means of agarase, the agarose may also be liquefied by cyclic heating of the sample for example during the PCR process.

20 It goes without saying that the exemplary embodiments described here are not limited to agarose as hydrogel, it also being possible to use other hydrogels with the desired properties. For example, a hydrogel based on collagen, polyacrylamide or similar substances would be possible

25 here. The receiving units may also assume different forms, depending on the desired application.

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